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(54) Title: RECESSIVE PLANT VIRAL RESISTANCE RESULTS FROM MUTATIONS IN TRANSLATION INITIATION FACTOR eIF4E

(57) Abstract: The present invention relates to methods of imparting virus resistance to plants. In one aspect, this method involves silencing a gene encoding a translation initiation factor eIF4E in the plant. In another aspect, this method involves overexpressing a heterologous translation initation factor eIF4E in a plant. The present invention further relates to a genetic construct containing a nucleic acid molecule encoding a heterologous translation initiation factor eIF4E, as well as to an expression system containing the genetic construct and a host cell transformed with the genetic construct. The present invention also relates to transgenic plants, seeds, and plant parts transformed with the genetic construct. The present invention also relates to an isolated nucleic acid molecule encoding a mutant translation initiation factor eIF4E that is effective in imparting virus resistance in plants. The present invention also relates to a mutant translation initiation factor eIF4E and a method for making the mutant.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/40184

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01H 5/00, 5/08, 5/10; C12N 5/04, 15/09, 15/29, 15/70, 15/82, 15/87, 15/90
US CL : 435/320.1, 419, 468, 471; 536/23.6; 800/278, 279, 285, 286, 295, 298

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/320.1, 419, 468, 471; 536/23.6; 800/278, 279, 285, 286, 295, 298

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, Agricola, CAplus, Biosis, EMBL, GenBank, EST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LELLIS et al. Loss-of-Susceptibility Mutants of Arabidopsis thaliana Reveal an Essential Role for eIF(iso)4E During Potyvirus Infection. Curr. Biol.. 25 June 2002, Vol. 12, pages 1046-1051, see whole document.	1-8, 12-33, 35-41, 44-49
Y	SCHAAD et al., Strain-Specific Interaction of the Tobacco Etch Virus Nla Protein with the Translation Initiation Factor eIF4E in the Yeast Two-Hybrid System. Virology. 2000, Vol. 273, pages 300-306, see pages 300-303.	1-8, 12-33, 35-41, 44-49
Y	WITTMANN et al. Interaction of the Viral Protein Genome Linked of Turnip Mosaic Potyvirus with the Translational Eukaryotic Initiation Factor (iso) 4E of Arabidopsis thaliana Using the Yeast Two-Hybrid System. Virology. 1997, Vol. 234, pages 84-92, see pages 86-89.	1-8, 12-20, 44-49
A, T	YOSHII et al. The Arabidopsis Cucumovirus Multiplication 1 and 2 Loci Encode Translation Initiation Factors 4E and 4G. J. Virol. June 2004, Vol. 78, No. 12, pages 6102-6111, see pages 6104-6108.	1-5, 7, 8, 13-32

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GALLIE, D. R. Cap-Independent Translation Conferred by the 5' Leader of Tobacco Etch Virus Is Eukaryotic Initiation Factor 4G Dependent. J. Virol. December 2001, Vol. 75, No. 24, pages 12141-12152, see pages 12143-12146.	1-20, 44-49
Y	LEONARD et al. Complex Formation Between Potyvirus VPg and Translation Eukaryotic Initiation Factor 4E Correlates with Virus Infectivity. J. Virol. September 2000, Vol. 74, No. 17, pages 7730-7737, see pages 7732-7735.	1-8, 12-32, 44-49
A, T	NICAISE et al. The Eukaryotic Translation Initiation Factor 4E Controls Lettuce Susceptibility to the Potyvirus Lettuce mosaic virus. Plant Physiol. July 2003, Vol. 132, pages 1272-1282, see pages 1273-1274, 1276-1278.	1-8, 12-32, 44-49
X --- Y	DUPRAT et al. The Arabidopsis Eukaryotic Initiation Factor (iso)4E is Dispensable for Plant Growth but Required for Susceptibility to Potyviruses. Plant J. 2002, Vol. 32, pages 927-934, see whole document.	1, 2, 7, 8, 21-32 --- 3-6, 12-33, 35-41, 44-49
X --- Y	RUFFEL et al. A Natural Recessive Resistance Gene Against Potato Virus Y in Pepper Corresponds to the Eukaryotic Initiation Factor 4E (eIF4E). Plant J. 2002, Vol. 32, pages 1067-1075, see whole document.	21-33, 35-39 --- 1-8, 12-20, 40, 41, 44-49
A, T	WO 03/066900 A2 (GENOPLANTE-VALOR) 14 August 2003 (14.08.2003), Abstract	1-8, 12-33, 35-41, 44-49

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INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-33, 35-41, and 44-49 drawn to a first method of imparting virus resistance to plants comprising silencing a gene encoding a translation initiation factor eIF4E in the plant; and a first product, a genetic construct comprising a nucleic acid molecule which silences a gene encoding a translation initiation factor eIF4E in a plant, an expression system, host cell, and plant containing said genetic construct.

Group II, claim(s) 34, drawn to a second product, an isolated mutant translation initiation factor eIF4E.

Group III, claim(s) 50-58, drawn to a second method, of imparting virus resistance to plants comprising providing a nucleic acid molecule encoding a heterologous translation initiation factor eIF4E comprising an amino acid sequence of SEQ ID NO: 4 and variants at least 95% similar to SEQ ID NO: 4, wherein the mutant eIF4E is overexpressed.

Group IV, claim(s) 50-58, drawn to a third method, of imparting virus resistance to plants comprising providing a nucleic acid molecule encoding a heterologous translation initiation factor eIF4E comprising an amino acid sequence of SEQ ID NO: 6 and variants at least 95% similar to SEQ ID NO: 4, wherein the mutant eIF4E is overexpressed.

Group V, claim(s) 50-58, drawn to an fourth method, of imparting virus resistance to plants comprising providing a nucleic acid molecule encoding a heterologous translation initiation factor eIF4E comprising an amino acid sequence of SEQ ID NO: 8 and variants at least 95% similar to SEQ ID NO: 8, wherein the mutant eIF4E is overexpressed.

Group VI, claim(s) 50-58, drawn to a fifth method, of imparting virus resistance to plants comprising providing a nucleic acid molecule encoding a heterologous translation initiation factor eIF4E comprising an amino acid sequence at least 85% similar to a non-mutant initiation factor of SEQ ID NO: 2 and contains at least one substitution of at least one amino acid residue of SEQ ID NO: 2 selected from T51A, P66T, V67E, K71R, L79R, G107P, and D109R, wherein the mutant eIF4E is overexpressed.

Group VII, claim(s) 42 and 43, drawn to a sixth method, of making a mutant translation initiation factor eIF4E.

The inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of Group I is the silencing of a gene encoding eIF4E in plants to impart virus resistance. This special technical feature of Group I is not shared with the other groups. Group II is drawn to proteins that are not shared with the other groups. Groups III-VI are drawn to methods for imparting virus resistance to a plant comprising overexpressing a mutant eIF4E. The special technical feature of silencing a gene encoding eIF4E of Group I is not shared with the overexpression of a mutant eIF4E of the methods of Groups III-VI. Each of Groups III-VI encompass expressing different nucleotide sequences that are not shared with one another, making the methods distinct from one another. Group VII is directed to a method of making a mutant protein, and forms a different category of invention.

Applicants are reminded that different nucleotide sequences are structurally distinct chemical compounds. These sequences are thus deemed to normally constitute distinct inventions. Applicant is required to choose one sequence from the following group for searching with Group I: A) SEQ ID NO: 1; B) nucleotide sequences encoding SEQ ID NO: 4; C) nucleotide sequences encoding SEQ ID NO: 6; D) nucleotide sequences encoding SEQ ID NO: 8; E) nucleotide sequences encoding SEQ ID NO: 2 and containing at least one substitution of at least one amino acid residue of SEQ ID NO: 2 selected from the group consisting of T51A, P66T, V67E, K71R, L79R, G107P, and D109R.